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High Dietary Fat and Selenium Concentrations Exert Tissue- and Glutathione Peroxidase 1–Dependent Impacts on Lipid Metabolism of Young-Adult Mice

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Background: Excessive dietary selenium (Se; 3 mg/kg) or fat (>25%) intakes and overproduction of glutathione peroxidase 1 (GPX1) adversely affect body lipid metabolism.

Objective: The objective was to reveal impacts and mechanisms of a moderately high Se and a high fat intake on lipid metabolism in *Gpx1* knockout (KO) and wild-type (WT) mice.

Methods: The KO and WT mice (males, 12-wk-old, body weight = 24.8 ± 0.703 g) were allotted to 4 groups each ($n = 5$) and fed a sucrose-torula yeast basal diet (5% corn oil) supplemented with 0.3 or 1.0 mg (+Se) Se/kg (as sodium selenite) and 0% or 25% [high-fat (HF)] lard for 6 wk. Multiple physiological and molecular biomarkers (68) related to lipid metabolism and selenogenome expression in plasma, liver, and/or adipose tissue were analyzed by 2-way (+Se by HF) ANOVA.

Results: Compared with the control diet, the +Se diet decreased ($P < 0.05$) body-weight gain and plasma and liver concentrations of lipids (22–66%) but elevated (≤1.5-fold, $P < 0.05$) adipose tissue concentrations of lipids in the WT mice. The +Se diet up- and downregulated ($P < 0.05$) mRNA and/or protein concentrations of factors related to lipogenesis, selenogenome, and transcription, stress, and cell cycle in the liver (26% to 176-fold) and adipose tissues (14% to 1-fold), respectively, compared with the control diet in the WT mice. Many of these +Se diet effects were different ($P < 0.05$) from those of the HF diet and were eliminated or altered ($P < 0.05$) by the KO.

Conclusions: The +Se and HF diets exerted tissue-specific and GPX1 expression–dependent impacts on lipid metabolism and related gene expression in the young-adult mice. Our findings will help reveal metabolic potential and underlying mechanisms of supplementing moderately high Se to subjects with HF intakes. *J Nutr* 2020;150:1738–1748.

Keywords: lipogenesis, metabolism, mice, selenium, transcription

Introduction

It is well known that high dietary fat intakes impair body lipid metabolism and induce type 2 diabetes and obesity (1, 2). Intriguingly, we were able to induce gestational diabetes in pregnant rats and insulin resistance in their offspring by feeding them an excessively high selenium (Se) diet (3 mg/kg) (3). Similar detrimental potential of such an excessive intake of Se in causing hyperinsulinemia and insulin resistance was shown in 2 subsequent pig studies (4, 5). Meanwhile, data from human studies indicated a positive association between elevated body Se status and risks of hyperglycemia and type 2 diabetes (6–8). Mechanistically, the lipid accumulation and fatty acid profile changes in the liver and adipose tissue of pigs mediated by the high Se intake (3 mg/kg) concurred with elevated Se-dependent glutathione peroxidase (GPX) 1 (GPX1) activity in the tissues (9). In fact, overexpression of GPX1 induced type 2 diabetes–like phenotypes including elevated plasma and tissue lipid concentrations in mice at the age of 6 mo (10). In contrast, knockout (KO) of GPX1 and dietary Se deficiency were shown to be protective against the high-fat (HF) or Se intakes and/or the overproduced GPX1 activity–induced disorders of lipid metabolism (11, 12). In addition to GPX1, expressions of GPX3, selenoprotein P (SELENOP), and key genes in regulating lipogenesis, lipolysis, and protein synthesis were also altered by the high Se intake (3 mg/kg) in the liver, adipose tissue, and/or muscle of pigs (9).

Because high intakes of dietary Se and fat induced similar metabolic disorders, we speculated that shared mechanisms existed for their effects on Se and lipid metabolism. In fact, the HF diet–induced obesity in pigs was associated with altered tissue expressions of multiple selenoproteins involved in cell cycle, mitochondrial biogenesis, cell proliferation, and cell–cell interaction (13–15). Whereas HF intakes altered gene expressions related to stress [C-Jun proto-oncogene (*C-JUN*), c-Jun N-terminal kinase (*JNK*), p38 mitogen-activated protein kinase (*P38 MAPK*), mitochondrial biogenesis and fatty acid

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metabolism (16), and cell cycle, such as cyclin G1 (*Ccng1*) (17)] high Se (3 mg/kg) intakes showed similar effects on cancer cell proliferation (18) and fatty acid profiles (9). The metabolic consequences of the high Se (3 mg/kg) and HF intakes could seemingly result from mediating those common pathways. Although combined high intakes of both dietary fat and Se are not uncommon by the US public (19), previous studies seldom compared their singular and interaction effects directly or simultaneously on their potentially common key effectors or mediators. Another drawback of past research on the diabetogenic potential of Se (3, 5, 20, 21) was the use of extremely high Se concentrations (~3 mg/kg), which were ~10-fold the nutrient requirement of the modeled animals. While the recommended dietary allowance for Se is 55 μg/d for both male and female adults (22), the actual average daily Se intake from foods and supplements is 151 μg in males and 108 μg in females (23). Because the differences between the actual daily intakes and the recommendations are only 2- to 3-fold, a moderately high Se intake (e.g., 1 mg/kg) should be appropriate to be used in animal studies to simulate human cases. Furthermore, the above-postulated shared mechanisms between the high Se (3 mg/kg) and HF intakes on Se and lipid metabolism may be mediated by transcriptional regulation of selenoprotein gene expression (24). In fact, many observed responses of selenoprotein gene expression in different tissues to dietary Se deficiency or excess (25) could not be fully explained by mechanisms already tested. Thus, exploring such transcriptional regulation by dietary Se and fat intakes will not only help toward understanding their metabolic mechanism but also advance Se biology.

To address the aforementioned issues, we prepared 4 experimental diets with 2 concentrations of dietary Se (0.3 vs. 1 mg/kg) and fat (5% corn oil vs. 5% corn oil + 25% lard) each. Because of the illustrated roles of GPX1 in the Se and lipid metabolism (10, 26), we fed the KO and their wild-type (WT) mice with these 4 diets for 6 wk. Our objectives were to determine the following: *1*) whether the moderately high Se diet (1.0 mg Se/kg diet) produced hyperlipidemic effects similar to those of the HF diet, *2*) whether effects of these 2 diets varied with the GPX1 genotype and tissues, and *3*) whether transcriptional regulation was part of the mechanism for the effects of these diets.

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Supplemental Figures 1–3 and Supplemental Tables 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: *Actb*, B-actin; *Ccng1*, cyclin G1; *Cd36*, cluster of differentiation 36; C-JUN, C-Jun proto-oncogene; *Cpt1*, carnitine palmitoyltransferase 1; *Dio1–3*, iodothyronine deiodinase, types 1–3; *Elovl1–6*, elongation of very-long-chain fatty acid proteins 1–6; *Fads1*, fatty acid desaturase 1; *Fasn*, fatty acid synthase; *Fatp*, fatty acid transport protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPX, glutathione peroxidase; *Gpx4*, glutathione peroxidase 4; *Gr*, glucocorticoid receptor; *Hes1*, hairy and enhancer of split-1; HF, high-fat; *Hoxa5*, homeobox A5; JNK, c-Jun N-terminal kinase; *Klhdc1*, Kelch domain containing 1; KO, knockout; *Myod*, myogenic differentiation 1; NEFA, nonesterified fatty acid; *Nf1*, Neurofibromin 1; *Pgc1*, peroxisome proliferator-activated receptor γ coactivator 1; P38, p38 mitogen-activated protein kinase; P65, NF-κB p65 subunit; *Scd1*, stearoyl-CoA desaturase-1; Se, selenium; SELENOF, selenoprotein 15; *Selenoh*– *Selenox*, selenoproteins H–X; SREBP1, sterol regulatory element-binding protein 1; TC, total cholesterol; TF, transcription factor; TG, triglyceride.

Methods

Animals, diets, and managements

Our animal protocol was approved by the Institutional Animal Care and Use Committee of Cornell University. The genetic background of all mice was 129/SVJ × C57BL/6 (27), and the feeding and housing conditions were the same as previously described (12). A total of 20 WT and KO mice each (males, 12-wk-old, body weight = 24.8 ± 0.703 g) were divided into 4 groups (n = 5/group by genotype) and fed a sucrose-yeast meal basal diet (control; containing 0.3 mg Se/kg as sodium selenite and 5% corn oil; **Supplemental Table 1**) or the control + 0.7 mg Se/kg (+Se; 1.0 mg Se/kg and 5% corn oil) + 25% lard (Dyets, Bethlehem, PA) (HF; 0.3 mg Se/kg, 5% corn oil, and 25% lard) or both (HF+Se; 1 mg Se/kg, 5% corn oil, and 25% lard) for 6 wk. Before the experiment, all mice were fed an Se-adequate diet (0.3 mg/kg) and were grouped into these 4 dietary treatments based on age, litter, and body weight. Individual body weights of mice were recorded weekly.

Biochemical analyses of plasma and tissue samples

Whole blood of individual mice were collected at weeks 0 and 6 after overnight feed deprivation (8 h) to prepare plasma samples as previously described (3). Plasma concentrations of total triglyceride (TG), total cholesterol (TC), nonesterified fatty acid (NEFA), and glucose were determined as previously described (26). At the end of the experiment (week 6), all mice were killed after overnight (8-h) feed deprivation (5) to collect samples of liver and adipose tissue (retroperitoneal fat) (5). The samples were snap-frozen in liquid nitrogen and stored in a −80°C freezer until analyses. Total lipid was extracted from liver (50 mg) and adipose tissue (50 mg) for the analyses of TG, TC, and NEFA concentrations (28). Total lipids were extracted from the liver (250 mg) and adipose tissue (50 mg) (29) for the subsequent fatty acid analyses using GC (HP 6890; Hewlett Packard).

Putative transcription factor binding sites

The proximal promoter regions of selected selenoprotein genes, defined as −1000 bp upstream of the transcription start site of the selected genes of selenoproteins, were retrieved from the NIH/National Center for Biotechnology Information (NCBI) Entrez Gene database (http://www.ncbi.nlm.nih.gov/gene). The transcription factor (TF) binding sites were predicted using the Transcription Element Search System (TESS; http://www.cbil.upenn.edu/cgi-bin/tess/tess).

Real-time qPCR, and immunoblotting

Total mRNA was isolated from livers (20 mg) and adipose tissue (100 mg), and the subsequent quantification was carried out by real-time qPCR (7900 HT; Applied Biosystems) of key genes involved in lipid and fatty acid metabolism, and selected selenoprotein genes and their transcription factors predicted targeting to the proximal promoter regions of these genes as previously described (26). The primer sequences used for all the assayed genes are presented in **Supplemental Table 2**. The 2^{-ddCt} method was used for the quantification, with B-actin (*Actb*) as a reference gene, and the relative abundance was normalized to the control (as 1). The validity of *Actb* as the reference was verified by its amplification plots and dissociation curves of all samples from the liver and adipose samples (**Supplemental Figure 1**). Western blot analyses of key factors in the pathways of lipid metabolism and selected selenoproteins were performed as previously described (26), and the relative density of the protein bands were quantified by using ImageJ software (NIH) and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the loading control. The primary antibodies used for the Western blot analyses are presented in **Supplemental Table 3**.

Statistical analyses

Main effects of diets were analyzed using 2-way ANOVA (2 concentrations of dietary Se by 2 concentrations of dietary fat) within each genotype (SAS Institute, Inc.). Treatment mean differences were compared using Duncan's multiple-range test. Data are presented as means ± SEs, and the significance level was set at $P < 0.05$. The

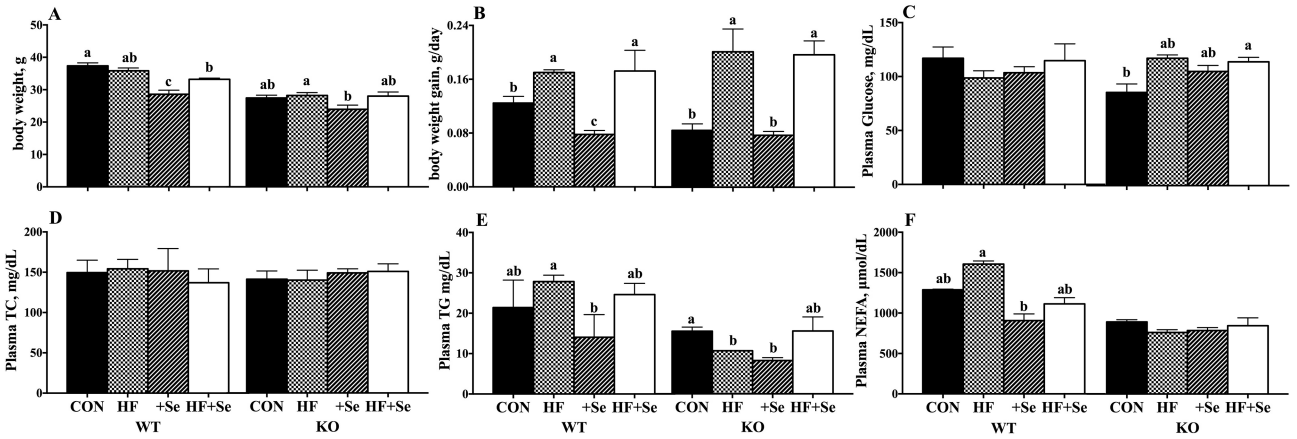

FIGURE 1 Body weight (A), daily body-weight gain (B), and plasma glucose (C), TC (D), TG (E), and NEFA (F) concentrations in WT and KO mice fed diets varying in fat and Se concentrations for 6 wk. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. Values are means ± SEs, $n = 5$. Bars without a common letter differ, $P < 0.05$. CON, control; HF, high-fat; KO, knockout; NEFA, nonesterified fatty acid; Se, selenium; TC, total cholesterol; TG, total triglyceride; WT, wild-type.

Pearson's correlation coefficient analysis was applied to assess the relation between different groups of measures (SAS Institute, Inc.), and only correlations with $R^2 > 0.8$, $P < 0.05$, are shown. Before these analyses, equal variances of variables were checked. The effects of genotype and its interactions with dietary Se and fat intakes were not directly analyzed in a 3-factor ANOVA due to the complexity of the 3-way interactions to be interpreted, our focus on the comparison of dietary Se and fat, and the distinction between the 2 genotypes in the overall responses and regression analysis (clusters).

Results

The initial plasma glucose, TC, TG, and NEFA concentrations and body weights of mice are presented in **Supplemental Figure 2**. The +Se diet group had a lower ($P < 0.05$) final body weight and daily gain of body weight compared with the other 3 diet groups (control, HF, and HF+Se) in the WT mice (**Figure 1**A, B). The HF and HF+Se diets produced greater ($P < 0.05$) daily gains of body weights than the other 2 diets in both genotypes. The HF+Se diet produced higher ($P < 0.05$) plasma concentrations of glucose than the control diet in the KO mice (Figure 1C). The +Se diet resulted in lower ($P < 0.05$) plasma concentrations of TG and NEFA than the HF diet in the WT mice, whereas both diets decreased ($P < 0.05$) plasma concentrations of TG in the KO mice compared with the control diet (Figure 1E, F). Dietary Se and fat concentrations showed no interaction effects on all these measures except for plasma concentrations of TG in the KO mice.

In the liver of WT mice, the +Se diet decreased ($P < 0.05$) TC and NEFA concentrations, whereas the HF diet enhanced ($P < 0.05$) TG and NEFA concentrations compared with the control diet (**Figure 2**A–C). The HF+Se diet also elevated ($P < 0.05$) hepatic TG and NEFA concentrations compared with the control diet in the WT mice. In contrast, the HF+Se diet led to lower ($P < 0.05$) concentrations of hepatic TG and NEFA than the control diet in the KO mice. In the adipose tissue of WT mice, the +Se diet elevated ($P < 0.05$) concentrations of TC, TG, and NEFA compared with the other diets. In the adipose of KO mice, the HF+Se diet resulted in consistent and substantial (>2-fold, $P < 0.05$) elevations of TC, TG, and NEFA

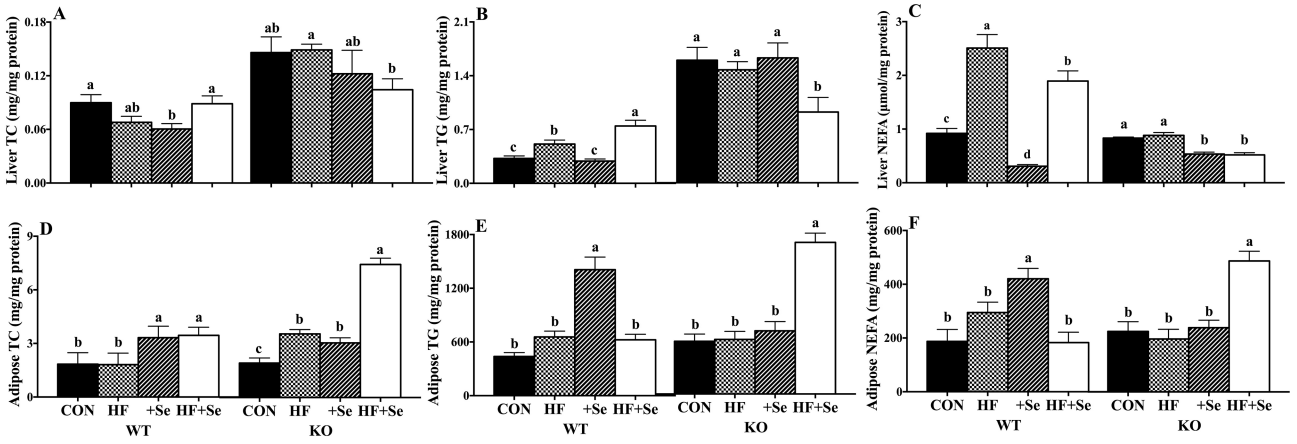

FIGURE 2 Hepatic concentrations of TC (A), TG (B), and NEFA (C) and adipose tissue concentrations of TC (D), TG (E), and NEFA (F) in WT and KO mice fed diets varying in fat and Se concentrations for 6 wk. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. Values are means ± SEs, $n = 5$. Bars without a common letter differ, $P < 0.05$. CON, control; HF, high-fat; KO, knockout; NEFA, nonesterified fatty acid; Se, selenium; TC, total cholesterol; TG, triglyceride; WT, wild-type.

TABLE 1 Free fatty acid profiles in the tissues of WT and KO mice fed diets varying in fat and Se concentrations for 6 wk[1]

	CON	HF	+Se	HF+Se
WT liver, mg/g				
Myristic acid (C14:0)	0.19 ± 0.020^{a}	0.17 ± 0.010^{a}	0.090 ± 0.010^{b}	0.17 ± 0.010^{a}
Palmitic acid (C16:0)	9.8 ± 0.52^{a}	10 ± 0.24^{a}	6.7 ± 0.45^{b}	6.5 ± 0.30^{b}
Palmitoleic acid [C16:1(n-7)]	1.9 ± 0.46^{a,b}	1.2 ± 0.050^{b}	0.34 ± 0.010^{c}	2.3 ± 0.31^{a}
Stearic acid (C18:0)	2.3 ± 0.060^{b}	2.7 ± 0.020^{a}	2.9 ± 0.10^{a}	2.1 ± 0.39^{b}
Oleic acid [C18:1(n-9)]	13 ± 0.52^{a}	14 ± 0.36^{a}	6.4 ± 0.39^{b}	12 ± 2.8^{a,b}
Linoleic acid [C18:2(n-6)]	13 ± 0.84^{a}	13 ± 0.36^{a}	5.8 ± 0.49^{b}	6.7 ± 3.3^{a,b}
γ-Linolenic acid [C18:3(n-6)]	0.20 ± 0.0030^{c}	0.32 ± 0.020^{b}	0.13 ± 0.010^{d}	1.5 ± 0.80^{a}
Paullinic acid [C20:1(n-7)]	0.26 ± 0.020^{b}	0.37 ± 0.010^{a}	0.13 ± 0.020^{c}	0.19 ± 0.070^{b,c}
Dihomo-γ-linolenic acid [C20:3(n-6)]	0.12 ± 0.010^{b}	0.15 ± 0.010^{b}	0.25 ± 0.020^{a}	0.54 ± 0.34^{a}
Behenic acid (C22:0)	2.2 ± 0.070^{b}	2.3 ± 0.13^{a,b}	2.9 ± 0.12^{a}	3.1 ± 0.18^{a}
DHA [C22:6(n-3)]	1.3 ± 0.060^{b}	1.4 ± 0.050^{a,b}	1.6 ± 0.030^{a}	2.0 ± 0.34^{a}
KO liver, mg/g				
Caprylic acid (C8:0)	7.1 ± 0.30^{a,b}	7.7 ± 0.20^{a}	7.0 ± 0.47^{a,b}	6.6 ± 0.18^{b}
Myristic acid (C14:0)	0.20 ± 0.020^{a}	0.17 ± 0.010^{a}	0.19 ± 0.020^{a}	0 ± 0^{b}
Palmitic acid (C16:0)	9.8 ± 0.49^{a}	8.8 ± 0.56^{a}	9.1 ± 0.14^{a}	5.5 ± 0.23^{b}
Palmitoleic acid [C16:1 (n-7)]	2.0 ± 0.12^{a}	0.27 ± 0.030^{b}	0.17 ± 0.010^{b}	0.34 ± 0.080^{b}
Oleic acid [C18:1(n-9)]	13 ± 0.59^{a}	10 ± 0.82^{a}	13 ± 0.68^{a}	6.8 ± 0.71^{b}
Linoleic acid [C18:2(n-6)]	11 ± 0.070^{a}	10 ± 1.0^{a}	9.0 ± 0.14^{b}	5.4 ± 0.36^{c}
γ-Linolenic acid [C18:3(n-6)]	0.16 ± 0.010^{a}	0.17 ± 0.010^{a}	0.16 ± 0.010^{a}	0 ± 0^{b}
Dihomo-γ-linolenic acid [C20:3(n-6)]	0.16 ± 0.010^{b}	0.20 ± 0.010^{a,b}	0 ± 0^{c}	0.29 ± 0.020^{a}
Behenic acid (C22:0)	2.5 ± 0.090^{b}	2.6 ± 0.11^{b}	3.0 ± 0.050^{a}	3.0 ± 0.060^{a}
DHA [C22:6(n-3)]	2.0 ± 0.050^{b}	1.9 ± 0.090^{b}	2.4 ± 0.010^{a}	1.9 ± 0.070^{b}
WT adipose, mg/g				
Myristic acid (C14:0)	2.2 ± 0.060^{a}	1.3 ± 0.020^{b}	2.0 ± 0.15^{a}	1.8 ± 0.12^{a}
Palmitic acid (C16:0)	32 ± 1.1^{a}	22 ± 0.74^{b}	32 ± 2.1^{a}	29 ± 0.74^{a}
Palmitoleic acid [C16:1 (n-7)]	20 ± 0.82^{a}	11 ± 0.64^{c}	22 ± 2.2^{a}	14 ± 0.46^{b}
Stearic acid (C18:0)	2.4 ± 0.24^{b,c}	2.8 ± 0.20^{b}	2.0 ± 0.070^{c}	4.1 ± 0.38^{a}
Oleic acid [C18:1(n-9)]	74 ± 2.8^{b}	67 ± 1.7^{b}	82 ± 6.1^{a,b}	88 ± 2.4^{a}
Linoleic acid [C18:2(n-6)]	49 ± 3.7^{b}	58 ± 10^{a,b}	58 ± 3.2^{a,b}	70 ± 4.7^{a}
Paullinic acid [C20:1(n-7)]	1.6 ± 0.030^{b}	1.6 ± 0.10^{b}	2.0 ± 0.14^{a,b}	2.5 ± 0.15^{a}
KO adipose, mg/g				
Caprylic acid (C8:0)	52 ± 1.8^{b}	59 ± 1.3^{a}	49 ± 1.2^{b}	50 ± 0.83^{b}
Myristic acid (C14:0)	2.2 ± 0.070^{a}	1.5 ± 0.06^{b}	2.1 ± 0.14^{a}	2.2 ± 0.18^{a}
Palmitic acid (C16:0)	32 ± 1.1^{b}	26 ± 0.69^{c}	29 ± 0.78^{b,c}	51 ± 5.0^{a}
Palmitoleic acid [C16:1 (n-7)]	21 ± 1.1^{a}	11 ± 0.91^{c}	16 ± 1.1^{b}	18 ± 1.3^{a,b}
Stearic acid (C18:0)	2.9 ± 0.26^{b}	3.6 ± 0.35^{a,b}	2.6 ± 0.32^{b}	5.4 ± 0.61^{a}
Oleic acid [C18:1(n-9)]	86. ± 2.4^{a}	73 ± 3.6^{b}	64 ± 2.2^{b}	95 ± 8.1^{a}
Linoleic acid [C18:2(n-6)]	53 ± 2.8^{b}	48 ± 2.1^{b,c}	42 ± 1.4^{c}	74 ± 4.1^{a}
Paullinic acid [C20:1(n-7)]	1.5 ± 0.040^{c}	1.7 ± 0.070^{b}	1.1 ± 0.070^{d}	3.0 ± 0.22^{a}

[1] Values are means ± SEs, $n = 5$. Labeled means in a row without a common letter differ, $P < 0.05$. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg, and HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. CON, control; HF, high-fat; KO, knockout; Se, selenium; WT, wild-type.

concentrations compared with the other 3 diets (Figure 2D–F). The HF and +Se diets produced different ($P < 0.05$) concentrations of all detected fatty acids, except for stearic acid (C18:0) , behenic acid (C22:0), and DHA [C22:6(n-3)] in the liver of the WT mice (Table 1). However, those differences between these 2 diets were seen in only 3 fatty acids {linoleic acid [LA, C18:2(n-6)], dihomo-γ-linolenic acid [C20:3(n-6)], and DHA} in the liver of the KO mice. In the adipose tissue, the HF and +Se diets produced different concentrations of 4 fatty acids each in the WT and KO mice, with 2 in common [myristic acid (C14:0) and palmitoleic acid (C16:1(n-7)] between the genotypes.

In the liver of WT mice (Figure 3A, **Supplemental Table 4**), the HF and +Se diets exerted similar upregulations (26% to 17-fold, $P < 0.05$) of mRNA levels of fatty acid elongases (*Elovl1*, *Elovl 3*, *Elovl 5*, and *Elovl6*) fatty acid desaturase 1 (*Fads1*), *Ccng1*, and stearoyl-CoA desaturase-1 (*Scd1*), and downregulations (13 to 46%, $P < 0.05$) of mRNA levels of

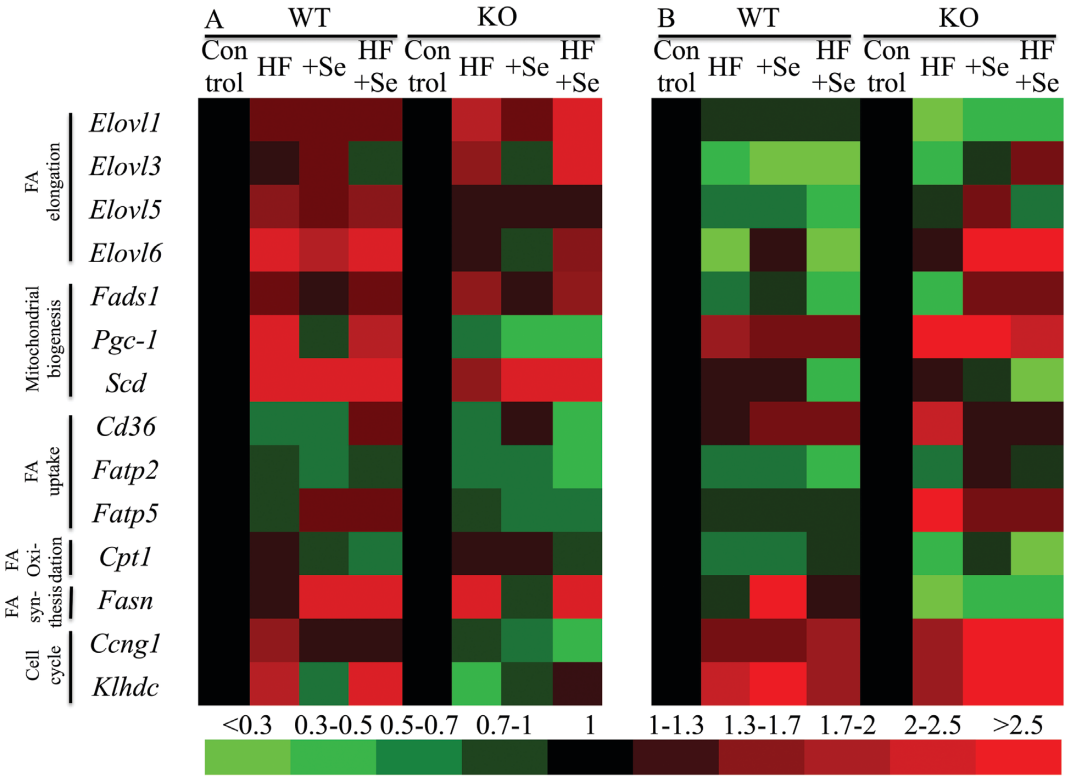

FIGURE 3 Relative mRNA levels of FA metabolism, cell cycle, and mitochondrial biogenesis pathways in the liver (A) and adipose tissue (B) of WT and KO mice fed diets varying in fat and Se concentrations for 6 wk. Relative gene expression is presented as log-2 fold-changes over the CON diet as a reference. Values between 0 and 1 (in green) indicate a decrease, whereas values >1 (in red) indicate an elevation in expression in the HF and/or +Se diet groups versus the CON diet group. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. *Ccng1*, cyclin G1; *Cd36*, cluster of differentiation 36; CON, control; *Cpt1*, carnitine palmitoyltransferase I; *Elovl1, 3, 6*, elongation of very-long-chain fatty acid proteins 1, 3, and 6; FA, fatty acid; *Fads1*, fatty acid desaturase 1; *Fasn*, fatty acid synthase; *Fatp2*, fatty acid transport protein 2; *Fatp5*, fatty acid transport protein 5; HF, high-fat; *Klhdc*, Kelch domain containing 1; KO, knockout; *Pgc-1*, peroxisome proliferator-activated receptor γ coactivator 1; *Scd1*, stearoyl-CoA desaturase-1; Se, selenium; WT, wild-type.

fatty acid transport protein (*Fatp*) 2 (*Fatp2*) and cluster of differentiation 36 (*Cd36*). The HF+Se diet showed effects on these genes similarly to those of the HF and +Se diets, with a few exceptions (*Elovl3* and *Cd36*), along with interaction effects of dietary fat and Se concentrations on carnitine palmitoyltransferase 1 (*Cpt1*) and Kelch domain containing 1 (*Klhdc1*) in the liver of the WT. In contrast, the HF and +Se diets exerted different effects ($P < 0.05$) on the mRNA levels of peroxisome proliferator-activated receptor γ coactivator 1 (*Pgc1*), *Cpt1*, *Klhdc1*, and *Fatp5* (10-49%) in the liver of WT mice. KO of *Gpx1* altered or reversed effects ($P < 0.05$) of the +Se diet on the mRNA levels of *Elovl3*, *Elovl6*, *Cd36*, *Fatp5*, *Cpt1*, fatty acid synthase (*Fasn*), and *Ccng1* and those of the HF diet on *Pgc1*, *Ccng1*, and *Klhdc1*, along with the interaction effects of the dietary Se and fat concentrations on *Cpt1* and *Klhdc1* compared with that shown in the liver of the WT mice.

Likewise, the HF and +Se diets produced similar elevations (10% to 1.7-fold, $P < 0.05$) of mRNA levels of *Pgc1*, *Scd1*, *Ccng1*, and *Klhdc1* and decreases in (14–94%, $P < 0.05$) mRNA levels of *Elovl1*, *Elovl3*, *Elovl5*, *Fads1*, *Fatp2*, *Fatp5*, and *Cpt1* in the adipose tissue of the WT mice (Figure 3B, Supplemental Table 4). Meanwhile, the + Se and HF diets exerted different or opposite impacts ($P < 0.05$) on the mRNA level of *Elovl6* and *Fasn* in the adipose tissue of the WT. There were interaction effects ($P < 0.05$) of dietary Se and fat concentrations on the mRNA levels of *Scd1*, *Cpt1*, and *Klhdc1* in the adipose tissue of the WT. KO of *Gpx1* altered or reversed effects ($P < 0.05$) of the + Se diet on *Cd36*, *Elovl5*, *Fads1*, *Scd1*, *Fatp2*, *Fatp5*, and *Fasn*, effects of the HF diet on *Elovl6* and *Fatp5*, or effects of the interaction between dietary Se and fat concentrations on *Cpt1* and *Klhdc1* in the adipose tissue of KO mice compared with in the WT.

In the liver of WT mice, the HF diet elevated (45% to 6-fold, $P < 0.05$) mRNA levels of 10 selenoproteins, whereas the +Se diet enhanced (41% to 3.7-fold, $P < 0.05$) *Selenov* and *Selenof* mRNA levels and decreased (55%, $P < 0.05$) iodothyronine deiodinase (*Dio*) type 2 (*Dio2*) mRNA levels (**Figure 4**A, **Supplemental Table 5**). In contrast, the +Se diet decreased (35–97%, $P < 0.05$) mRNA levels of 14 selenoproteins and the HF diet also decreased (38–88%, $P < 0.05$) those of 8 selenoproteins in the adipose tissue of WT (Figure 4B, Supplemental Table 5). Dietary Se and fat concentrations exerted interaction effects ($P < 0.05$) on mRNA levels of *Dio2*, *Dio3*, *Gpx4*, *Gpx6*, *Selenoi*, *Selenos*, and *Selenov* in the liver but not on any gene in the adipose tissue of WT mice. KO of *Gpx1* altered or reversed ($P < 0.05$) effects of the +Se diet on *Dio1*, *Gpx4*, *Gpx6*, *Selenoh*, *Selenoi*, *Selenok*, *Selenom*, *Selenos*, and *Selenox* and effects of the HF diet on *Gpx6*, *Dio1*, *Selenoh*, *Selenos*, *Selenoo*, and *Selenov* in the liver. KO of *Gpx1* also eliminated ($P < 0.05$) the interaction effects of dietary Se and fat concentrations on 6 selenoprotein genes (*Dio2*, *Dio3*, *Gpx4*, *Gpx6*, *Selenos*, and *Selenov*) and introduced new interaction

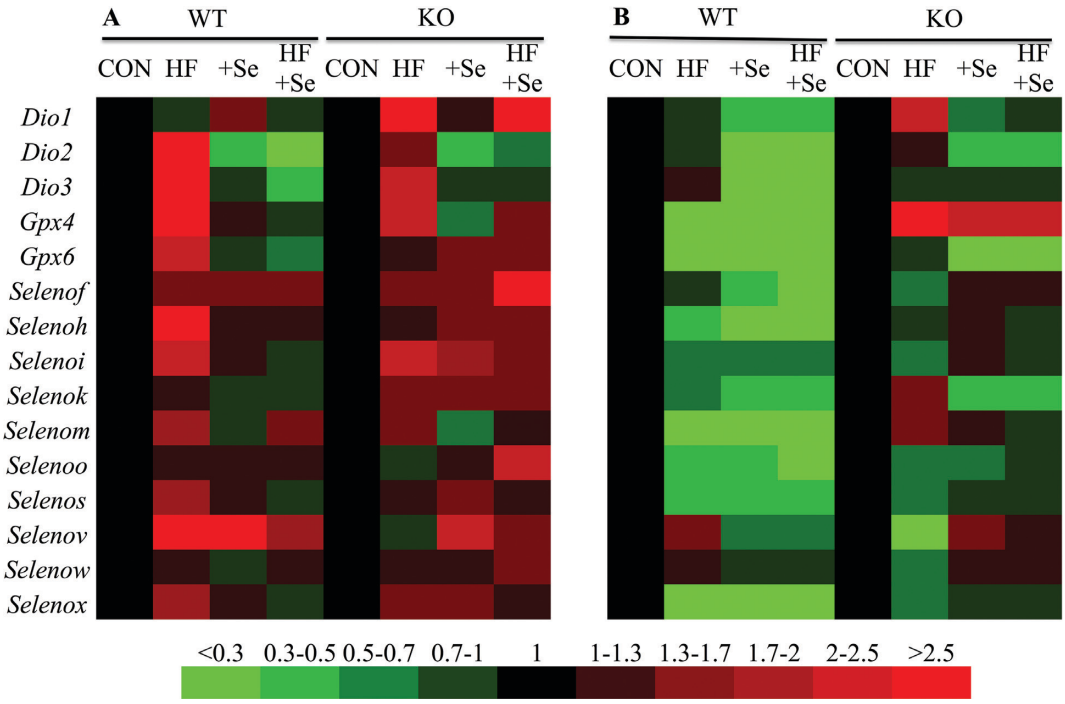

FIGURE 4 Relative mRNA levels of selenoproteins in the liver (A) and adipose tissue (B) of WT and KO mice fed diets varying in fat and Se concentrations for 6 wk. Relative gene expression is presented as fold-changes over the CON diet as a reference. Values between 0 and 1 (in green) indicate a decrease, whereas values >1 (in red) indicate an elevation in expression in the HF and/or +Se diet groups versus the CON diet group. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. CON, control; *Dio1-3*, iodothyronine deiodinases 1–3; *Gpx4*, 6, glutathione peroxidases 4, 6; HF, high-fat; KO, knockout; Se, selenium; *Selenof*, selenoprotein 15; *Selenoh*, selenoprotein H; *Selenoi*, selenoprotein I; *Selenok*, selenoprotein K; *Selenom*, selenoprotein M; *Selenoo*, selenoprotein O; *Selenos*, selenoprotein S; *Selenov*, selenoprotein V; *Selenow*, selenoprotein W; *Selenox*, selenoprotein X; WT, wild-type.

effects ($P < 0.05$) on *Selenoi* and *Selenoo* in the liver of KO mice compared with that in the WT mice. Likewise, KO of *Gpx1* altered or reversed ($P < 0.05$) effects of the +Se diet on *Dio3*, *Gpx4*, *Selenof*, *Selenoh*, *Selenoi*, *Selenom*, *Selenos*, *Selenov*, and *Selenox* and effects of the HF diet on *Dio1*, *Gpx4*, *Gpx6*, *Selenof*, *Selenoh*, *Selenok*, *Selenom*, *Selenov*, and *Selenow*, along with new interaction effects on *Selenoo* in the adipose tissue.

In the liver of WT mice, the +Se diet led to 87% to 4.3-fold higher mRNA levels ($P < 0.05$) of CCAAT-enhancer-binding protein B (*C/ebpb*) and glucocorticoid receptor (*Gr*) compared with the control diet (**Figure 5**A). Meanwhile, the HF diet enhanced mRNA levels ($P < 0.05$) (1.4 to 4-fold) of Hes family BHLH transcription factor 1 [hairy and enhancer of split-1 (*Hes1*)], neurofibromin 1 (*Nf1*) and myogenic differentiation 1 (*Myod*) and suppressed mRNA levels ($P < 0.05$, 97%) of homeobox a5 (*Hoxa5*) compared with the control diet. However, both diets decreased ($P < 0.05$, 55–97%) mRNA levels of *C/ebpb*, transcriptional repressor protein YY1 (*Yy1*), *Hoxa5*, *Gr*, *Hes1*, *Nf1*, and *Myod* in the adipose tissue of WT mice (Figure 5B). KO of *Gpx1* attenuated ($P < 0.05$) most of these changes by the diet treatments.

In the liver of WT, the HF and +Se diets caused similar elevations ($P < 0.05$) of GPX1 (59–71%) and GPX3 (41–62%) compared with the control diet (**Figure 6**A). However, the +Se diet increased ($P < 0.05$) SELENOP (116%), stress-related proteins (JNK, 116%; P38 MAPK, 176-fold), and transcription factor p65 [NF-κB p65 subunit (P65), 92-fold] and lowered ($P < 0.05$) C-JUN (54%) and sterol regulatory element-binding protein 1 (SREBP1; 99%). In contrast, the HF diet affected ($P < 0.05$) only C-JUN (+ 40%) and SREBP1 (+51%). The KO of *Gpx1* precluded or reversed ($P < 0.05$) the effects of the +Se and/or HF diet on all proteins in the liver of WT mice, with the exceptions of SREBP1 by the +Se diet and GPX3 by both diets (Figure 6B). There were interaction effects ($P < 0.05$) of dietary Se and fat concentrations on GPX1, SELENOF, and CCAAT/enhancer binding protein α (C/EBPA) in the liver of WT mice, but only on JNK in the liver of KO mice.

In the adipose tissue of WT mice, the HF and +Se diets increased ($P < 0.05$) SELENOP (1.2- to 1.4-fold), C-JUN (147- to 157-fold), and SREBP1 (78- to 92-fold) over the control diet (**Figure 7**A). Unlike the HF diet, the +Se diet also increased ($P < 0.05$) GPX3 (1.4-fold) and P38 MAPK (37%). KO of *Gpx1* precluded ($P < 0.05$) the induced elevations of SELENOP and P38 MAPK, but not SREBP1, by the +Se and/or HF diet in the adipose tissue of KO mice, compared with those in the WT mice (Figure 7B). There were interaction effects ($P < 0.05$) of dietary Se and fat concentrations on SREBP1 in the adipose tissue of both genotypes and C-JUN in the WT mice.

In the WT mice, there were stronger correlations ($R^2 > 0.8$) among the dietary concentrations of Se and fat and the gene expressions of 7 TFs and selenoproteins in the adipose tissue than in the liver (**Supplemental Figure 3**). Specifically, all 7 TFs were positively correlated with mRNA levels of 9 selenoprotein genes and SELENOP in the adipose tissue of WT mice. In contrast, only 3 TFs were correlated with the expression of 4 selenoprotein genes and GPX3 in the liver of WT mice. Similar poor correlations of these parameters were also seen in the liver of KO mice. However, there were 6 positive and 3 negative

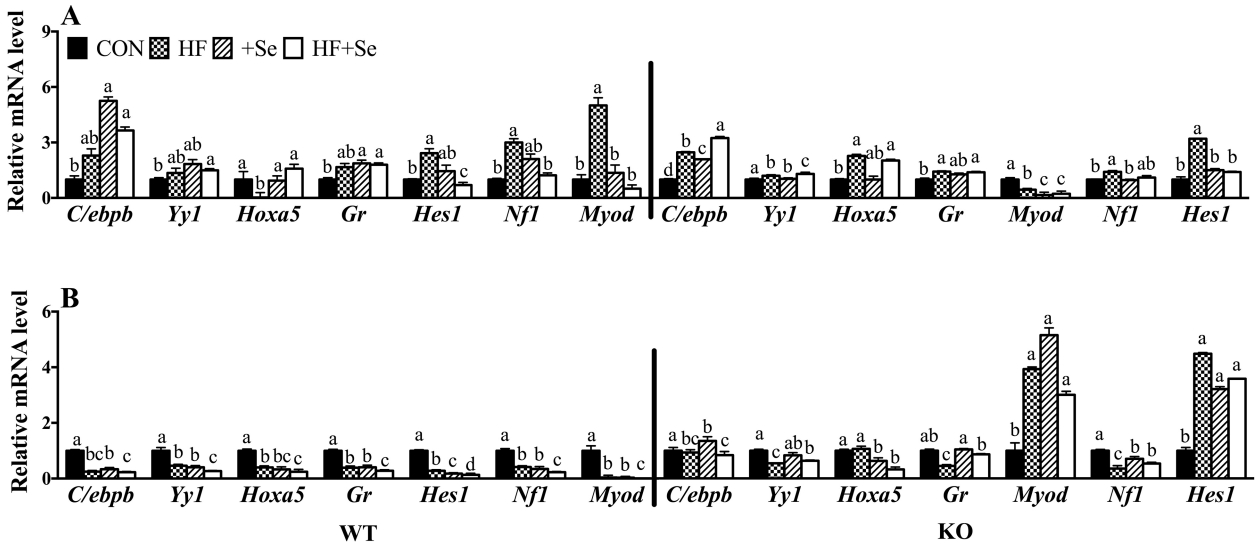

FIGURE 5 Relative mRNA levels of transcription factors of selenoprotein genes in the liver (A) and adipose tissue (B) of WT and KO mice fed diets varying in fat and Se concentrations for 6 wk. Relative gene expression is presented as log-2 fold-changes over the CON diet as a reference. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. Values are means ± SEs, $n = 5$. Bars without a common letter differ, $P < 0.05$. *C/ebpb*, CCAAT/enhancer binding protein B; CON, control; *Gr*, glucocorticoid receptor; *Hes1*, hairy and enhancer of split-1; HF, high-fat; *Hoxa5*, homeobox A5; KO, knockout; *Myod*, myogenic differentiation 1; *Nf1*, neurofibromin 1; Se, selenium; WT, wild-type; *Yy1*, transcriptional repressor protein YY1.

correlations between 5 TFs and 7 selenoprotein genes in the adipose tissue of KO mice.

Discussion

Most importantly, our study revealed a novel tissue-specific (liver vs. adipose) and GPX1 genotype–dependent (WT vs. KO) response to dietary Se and fat supplementation or overdosing by the young-adult mice. Phenotypically, the +Se diet decreased, whereas the HF diet increased, daily gain of body weight and plasma and liver TC, TG, or NEFA concentrations in the WT mice. Meanwhile, the +Se diet exerted opposite effects on the concentrations of lipids in the liver and adipose tissue. Intriguingly, the *Gpx1* KO attenuated, eliminated, or even reversed many of these diet effects. Comparatively, our observed benefits of feeding the +Se diet (1.0 mg Se/kg) in improving the metabolic phenotypes (30–32) were different from the adverse outcomes of feeding 3.0 mg Se/kg (9). Despite marginal differences in the initial body weights and plasma concentrations of glucose and NEFA between the treatment groups, the above-described different effects of +Se and HF and their dependence on tissue specificity and GPX1 expression in the present study should not have been confounded. Thus, our study integrates and extends previous research on singular testing of dietary Se and fat effects (9, 10, 12, 13) and offers a new view of the comparative roles of high dietary fat and moderately high Se (1 mg Se/kg) intakes in regulating body lipid metabolism (9, 33).

Despite different or opposite metabolic impacts, the +Se and the HF diets exerted similar effects on the mRNA levels of a majority of the assayed genes involved in lipid metabolism in the liver and adipose tissues of the WT mice. Apparently, the metabolic phenotype differences that resulted from these 2 diets were not mediated by systematic shifts in the major lipid metabolism pathways. Instead, those differences might be attributed to a few differentially regulated genes in the liver (*Pgc1*, *Cpt1*, *Fatp5*, and *Klhdc*) and the adipose tissue (*Elov6* and *Fasn*) by the 2 diets. Meanwhile, the +Se diet suppressed hepatic SREBP1 and elevated stress-related proteins of JNK, P38 MAPK, and P65. Among these genes and proteins, *Pgc1*, *Cpt1*, and *Fatp5* are involved in mitochondrial biogenesis, fatty acid oxidation, and fatty acid transportation (34–36). KLDHC may activate apoptosis signal-regulating kinase 1 (ASK1) to induce obesity (14, 37), and SREBP1 is a key regulator (stimulator) of lipogenesis (38). Although JNK and P38 MAPK were associated with the development of diabetes (39, 40), an elevated JNK and P38 MAPK were shown to improve energy homeostasis (41). Presumably, an attenuated fatty acid transportation could contribute to the decreased hepatic NEFA, 2 SFAs, 2 MUFAs, and LA in the WT mice fed the +Se diet (42). The suppressed mitochondrial biogenesis might be explained by a feedback mechanism of the decreased hepatic lipid accumulation for maintaining tissue energy homeostasis (43). Furthermore, the +Se diet–mediated changes in the hepatic fatty acid profiles might also help improve the metabolic phenotype. This was because decreases in SFAs and LA were supposed to limit their potential in inducing obesity and inflammation (44–46), whereas elevations in DHA could help prevent diabetes (47–50). Avoiding a high-MUFA diet (>38% of total energy) was also shown to be beneficial (51).

It was intriguing that the +Se diet suppressed the expression of essentially all assayed selenoprotein genes in the adipose tissue of WT mice, whereas the HF diet enhanced the expression of many selenoprotein genes in the liver of WT mice. Comparatively, regulations of selenoprotein gene expression in the liver by the +Se diet and in the adipose tissue by the HF diet were either less intensive or more variable. However, these tissue-dependent differential regulations of selenoprotein gene expression by the 2 diets were not consistent with their effects on the mRNA levels of the 7 TFs that could potentially bind the proximate promoters of the selenoprotein genes. These 2 diets suppressed the expression of all the 7 TFs in the adipose tissue

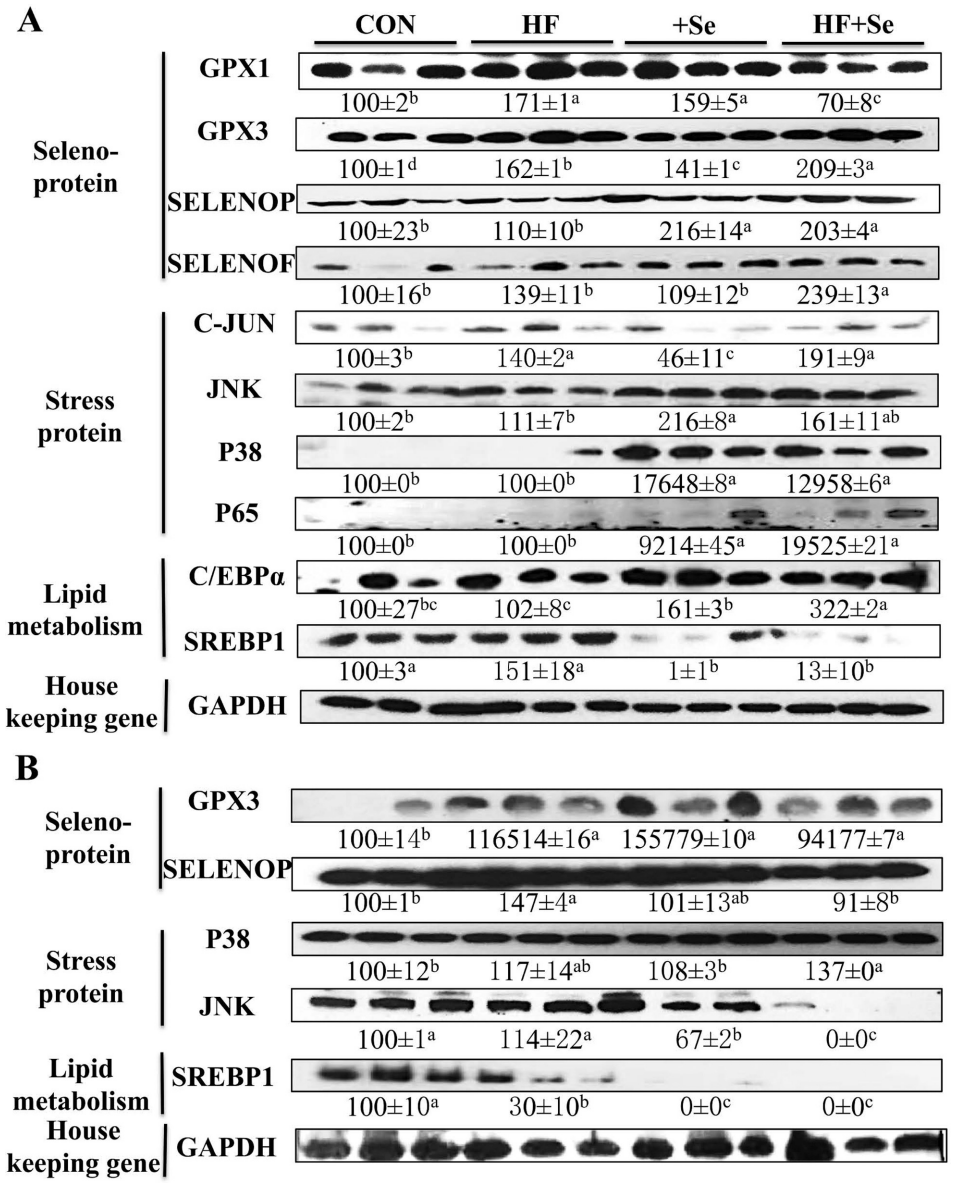

FIGURE 6 Relative protein concentrations of selenoproteins, stress proteins, and lipid metabolism pathways in the liver tissue of the WT (A) and KO (B) mice fed diets varying in fat and Se concentrations for 6 wk. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. Values below the bands are means ± SEs, $n = 3$. Values without a common superscript letter differ, $P < 0.05$. C/EBPα, CCAAT/enhancer binding protein α; C-JUN, C-Jun proto-oncogene; CON, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPX1, glutathione peroxidase 1; GPX3, glutathione peroxidase 3; HF, high-fat; JNK, c-Jun N-terminal kinase; KO, knockout; P38, p38 mitogen-activated protein kinase; P65, NF-κB p65 subunit; Se, selenium; SELENOF, selenoprotein 15; SELENOP, selenoprotein P; SREBP1, sterol regulatory element-binding protein 1; WT, wild-type.

of WT mice, but enhanced the expression of all these TFs in the liver, except for *Hoxa5*, which was downregulated by the HF diet. Apparently, it is novel to reveal the role of dietary Se and fat in regulating the gene expression of these TF in vivo, and a correlation between *Hoxa5* and fat distribution status (52) and thioredoxin reductase 1 mRNA expression (53) may lead to revealing a new pathway for dietary Se and fat intake in regulating body Se and lipid metabolism. However, the above-described tissue- and diet-dependent differential regulations of selenoprotein gene expression were either mediated by other TFs and/or mechanisms beyond transcription.

There were several clues to explain the distinct differences between the liver and adipose tissue in response to the +Se and HF diets. First, the +Se diet, as mentioned above, exerted very different or opposite impacts on the mRNA and protein levels of key factors involved in lipogenesis and lipolysis between the 2 tissues. A stark contrast was that the +Se diet diminished the protein production of the gate-keeping regulator of lipogenesis SREBP1 in the liver but elevated it by 78-fold in the adipose tissue. The induced lipid accumulation in the adipose tissue by the +Se diet may get worse after a longer feeding period and cause global lipid toxicity in mice (54) due to its association with the development of obesity and type 2 diabetes (55, 56). Second, these 2 diets produced relatively large differences in the mRNA levels of *Dio2*, *Gpx6*, *Selenom*, *Selenow*, and SELENOP in the liver and of *Dio3*, *Selenov*, *Selenow*, and GPX3 in the adipose tissue. Although elevated GPX1, GPX3, and SELENOP could promote obesity or lipid accumulation (10, 57, 58), suppressing

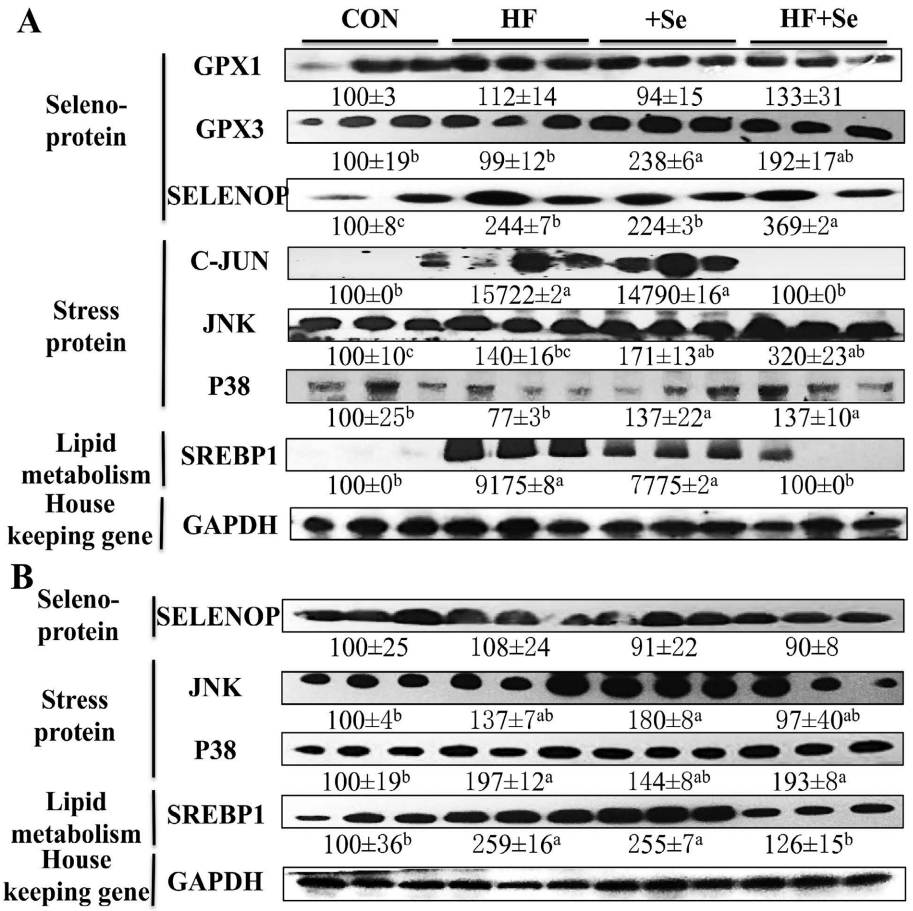

FIGURE 7 Relative protein concentrations of selenoproteins, stress proteins, and lipid metabolism pathways in the adipose tissue of the WT (A) and KO (B) mice fed diets varying in fat and Se concentrations for 6 wk. CON diet: 5% corn oil and 0.3 mg Se/kg; HF diet: CON diet + 25% lard; +Se diet: CON diet + 0.7 mg Se/kg; HF+Se diet: CON diet + 25% lard + 0.7 mg Se/kg. Values below the bands are means ± SEs, $n = 3$. Values without a common superscript letter differ, $P < 0.05$. C-JUN, C-Jun proto-oncogene; CON, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPX1, glutathione peroxidase 1; GPX3, glutathione peroxidase 3; JNK, c-Jun N-terminal kinase; KO, knockout; P38, p38 mitogen-activated protein kinase; Se, selenium; SELENOP, selenoprotein P; SREBP1, sterol regulatory element-binding protein 1; WT, wild-type.

functional expressions of *Dio2*, *Dio3*, *Gpx6*, *Selenom*, *Selenov*, and *Selenow* and/or maintaining a unique balance between them and GPX1, GPX3, and SELENOP might help explain how the +Se diet mediated its metabolic effects differently from the HF diet in the 2 tissues (57–64). Because DIO1 was reported to be negatively correlated with obesity (59), the suppressed expression of genes with similar functions (*Dio2*, *Dio3*) might promote lipid accumulation in the adipose tissue of WT mice (57–62). Last, the +Se diet did not decrease 2 SFAs, 2 MUFAs, and LA or elevate DHA in the adipose tissue. The lack of those changes, contrary to that observed in the liver, could contribute to the lipid accumulation in the adipose tissue (44–51).

KO of *Gpx1* altered, eliminated, or even reversed effects of the HF, +Se, and HF+Se diets on the mRNA levels of many assayed genes and related protein concentrations in the 2 tissues. This clearly illustrated a predominant role of GPX1 expression in controlling the responses of these genes and proteins to these 3 experimental diets. Recently, we unveiled a novel transcriptional inhibition mechanism (activator protein 1 and D-box binding PAR BZIP transcription factor) through controlling intracellular redox status by the GPX1 overproduction in downregulating the functional expression of a regenerating family protein, REG2 (65). In fact, similar importance of GPX1 expression (KO or overexpression) was shown in regulating responses of mice to dietary Se deficiency, adequacy, or excess (26, 66, 67). However, the KO effects in the present study were not universal across all genes or proteins. A good example was the lack of genotype differences in the responses of tissue GPX3 and SREBP1 to the HF and +Se diets. A new question is why or how the KO affected responses of certain genes or proteins to the dietary Se and fat intakes. Another question is how the KO did not block or reverse the metabolic impacts of the experimental diets despite the aforementioned capacity in altering the responses of related gene expression and protein production.

In summary, the present study demonstrated tissue-specific and GPX1 expression–dependent impacts of the +Se diet, with a moderately high dietary Se concentration (1.0 mg Se/kg), on body-weight gain, tissue lipid and fatty acid profiles, and related gene expression, protein production, and transcriptional regulation. These impacts were largely different from those of the HF diet (5% corn oil + 25% lard) or those associated with much higher dietary Se intakes (3 mg/kg) (9, 13). Our findings may add a new and integrated perspective to help assess and prevent potential risks of excessive Se supplementation to subjects with high intakes of dietary fat.

Acknowledgments

The authors' responsibilities were as follows—XGL: designed the research and had primary responsibility for the final content; ZZ, JK, and XGL: conducted the experiments and analyzed the data; ZZ and XGL: wrote the manuscript; and all authors: read and approved the final manuscript.

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